REMARKS

Claims 1-40 constitute the pending claims in the present application. Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

1-4. Applicants note that the previous amendment was entered and considered, and that claims 1-13 and 23-36 are withdrawn as being directed to a non-elected invention. Applicants will cancel such claims upon indication of allowable subject matter.

5. Applicants note that item BX on the previous IDS was not considered. This item will not be resubmitted.

6. Claims 14-22 and 37-40 are rejected under 35 U.S.C. § 101 because the claimed invention allegedly lacks patentable utility. Applicants respectfully traverse this rejection.

The second Advisory Action requests citations to places where the MPEP permits the use of publications in place of declarations under 37 C.F.R. 1.132. Perhaps the passage most relevant to the present situation is MPEP 2107. The penultimate paragraph of this section includes a statement that is quite clear on this point:

"The applicant [may rebut a *prima facie* showing of no specific and substantial credible utility] by ...providing evidence in the form of a declaration under 37 C.F.R. 1.132 or ***a patent or a printed publication*** that rebuts the basis or logic of the *prima facie* showing." (emphasis added)

Applicants are unable to determine any construction of this paragraph that would not require the Examiner to consider Faye et al. or other post-filing evidence, whether it is in the form of a declaration or not.

This passage, moreover, is entirely consistent with other related passages. For example, MPEP 2107.02 IV, second paragraph, states that "the examiner should provide documentary evidence ***regardless of the publication date*** ... to support the factual basis for the *prima facie* showing of no specific and substantial credible utility." (emphasis added) If the examiner may

rely on post-filing evidence to establish a lack of utility, certainly applicants must be permitted to use post-filing evidence to r but any asserted lack of utility.

MPEP 2107.02 VI also establishes the interchangeability of declarations and publications. "An applicant can [rebut the *prima facie* showing] using any combination of the following: amendments to the claims, arguments or reasoning, or new evidence submitted in an affadavit or declaration under 37 C.F.R. 1.132, or in a printed publication."

Applicants also respectfully remind the Examiner of the burden of proof on this issue, as stated in MPEP 2107.02 VII: "the applicant does not have to provide evidence sufficient to establish that an asserted utility is true 'beyond a reasonable doubt.' ... Nor must an applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty. ... Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true." (emphasis in original).

The Examiner's position appears to lack any evidentiary basis, whereas Applicants, *fully in compliance with procedures laid out in the MPEP*, have provided factual evidence demonstrating that the claimed subject matter possesses the utility asserted in the application. The assertions of the Examiner regarding *In re Brana*, even if true, are contradicted by clear protocols laid out in the MPEP. Reconsideration and withdrawal of this rejection are respectfully requested.

For the Examiner's convenience, Applicants provide herewith a translation of Faye et al. Applicants respectfully point out that it is irrelevant as to whether Faye et al. disclose a drug screening assay – the relevant disclosure is found throughout the present application. See, for example, page 7, lines 25-29, page 26, lines 11-28, page 29, lines 26-30, page 31, lines 10-17, etc. Applicants also respectfully point out that many other asserted uses of the claimed subject matter are described throughout the subject application. Faye et al. is cited merely to demonstrate that CAK1 in fact has the functions ascribed to it by Applicants, and that therefore this protein would be suitable for use in all the ways set forth in the present application.

The first Advisory Action states that Faye et al., discussed in detail below, has not been considered because it published after the priority date of the present application. Applicants

respectfully point out, however, that the law makes no such distinction, as considered in detail above. The case law and administrative documents discussed above and previously cited merely demonstrate that the availability of such evidence before the filing date of an application is not necessary for its consideration and successful rebuttal of a utility rejection.

Faye et al., WO 99/07836, cited as reference BV in the IDS filed January 29, 2001, discloses nucleotide and amino acid sequences with strong similarity to SEQ ID Nos. 13 and 14. For example, SEQ ID No. 2 of Faye et al. differs from SEQ ID No. 14 of the present application by merely six amino acids, 98% identity (see sequence comparison previously submitted as Exhibit A). On page 8, lines 30-36, Faye et al. state that cells transformed by a plasmid encoding SEQ ID No. 2 (CaCIV1) rescues *S. cerevisiae* cells which lack a functional ScCIV1 gene, a gene essential for cell viability. Accordingly, an agent which blocks the function of CaCIV1 would be expected to exhibit antifungal activity. Applicants asserted that the claimed subject matter was useful for drug screening assays on page 4, lines 5-6, and page 9, lines 11-18, and Faye et al. demonstrate the accuracy of these assertions.

The first Advisory Action further states that "the CAK1 utility in diagnostic tool has not been asserted in the specification." Applicants submit that use as a diagnostic tool, despite the Office Action's assertions to the contrary, is a *specific and substantial* utility that was asserted in the present application. The application asserts this utility at the top of page 4 and the first full paragraph on page 9, as well as the paragraph bridging pages 15 and 16 and lines 17-29 of page 25. The last of these passages points *specifically* to CAK1, SEQ ID No. 13, contrary to the Examiner's assertions in the first Advisory Action. The mere fact that other sequences may also be used for this purpose does not contradict this utility, or make it any less specific. For example, many different compounds have been identified – and patented – as antidepressant pharmaceuticals. The mere fact that *one* antidepressant is known does not make subsequent compounds identified as having similar activity any less useful, nor their utility any less specific, as the arguments recited in the Office Action suggest. Accordingly, the mere fact that other nucleic acids identified and sequenced from *C. albicans* could be used for diagnostic purposes does not undercut the fact that the presently claimed subject matter could also be used to diagnose the presence of *C. albicans* in a patient. Contrary to the statements in the Office Action, it is not true that *any* nucleic acid would have this utility. This utility is dependent on the

particular sequence disclosed by Applicants, and random sequences would typically be ineffective for this purpose. This fact demonstrates that the asserted utility is, in fact, specific.

For the reasons set forth above, Applicants submit that the pending claims fully comply with the requirements of 35 U.S.C. § 101. Reconsideration and withdrawal of this rejection is respectfully requested.

With respect to the rejection of these claims as not being enabled because the claimed invention was not allegedly supported by a utility, Applicants submit that the uses described above were described in the application with sufficient detail and clarity that one of skill in the art could have practiced the claimed invention throughout its scope. Reconsideration and withdrawal of this rejection is respectfully requested.

7. Claims 37-40 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent it is maintained over the claims as amended.

Applicants have amended claim 37 to more particularly point out conditions recited on page 13 of the application. Reconsideration and withdrawal of this rejection is respectfully requested.

8. Claims 37-40 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Applicants respectfully traverse this rejection to the extent it is maintained over the claims as amended.

Applicants have amended claim 37 as pointed out above. Applicants submit that hybridization is a common laboratory technique, and that one of skill in the art would readily be able to determine whether or not a nucleic acid sequence hybridizes to another under specified conditions. Reconsideration and withdrawal of this rejection is respectfully requested.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,

Date: March 11, 2002

David P. Halstead, Ph.D.
Reg. No. 44,735

Customer No: 28120
Docketing Specialist
Ropes & Gray
One International Place
Boston, MA 02110
Phone: 617-951-7000
Fax: 617-951-7050

EXHIBIT A

ACTIVATING KINASE FOR CYCLIN DEPENDENT PROTEIN KINASES, AND ITS USES

The present inv ntion relates to a new kinas from *Candida alibcans*, an activator of cyclin dependent protein kinases, and to its uses.

Cyclin dependent protein kinases (Cdk) are regulators of the cell division cycle in eukaryotes, essential both at the level of the G1/S transition and at the G2/M transition of the cell cycle. The CDC28 from *Saccharomyces cerevisiae* and the CDC2 from *Schizosaccharomyces pombe* are the first Cdks that have been identified.

The activation of Cdk necessitates both the attachment of a cyclin molecule, and the phosphorylation of the Cdk on a conserved threonine residue, situated in a region designated: "T loop".

It has been shown that this phosphorylation is conducted by a kinase called: "Cdk-activating kinase" (CAK), which, in vertebrates, is present in the form of a heterotrimer comprising a catalytic subunit designated Cdk7, a subunit of the cyclin type, called cyclin H, and a MAT-1 factor [for review, see SOLOMON, Trends Biochem. Sci. 19, 496-500 (1994)]. The Cdk7-cyclin H complex is also a component of the TFIIH complex, which is necessary for the basal transcription of genes by RNA polymerase II, and is involved in the phosphorylation of repeated sequences of the carboxy terminal domain (CTD) of the large subunit of this polymerase.

In the scissiparous yeast *Schizosaccharomyces pombe*, a complex similar to Cdk7-cyclin H, comprising a catalytic subunit designated Crk1, and a cyclin regulator designated Mcs2 have b en identified. It has been shown that the *Crk1*

gene was ss ntial for cell viability, and it has been observed *in vitro* that the Crk1-Mcs2 complex was associat d with CAK activity and CTD-kinase activity [BUCK et al., EMBO J., 14 (24), 6173-83 (1995); DAMAGNEZ et al., EMBO J., 14 (24), 6164-72 (1995)].

In the budding yeast *Saccharomyces cerevisiae*, a complex comprising a kinase (Kin28) and a cyclin (Ccl1) which are related, respectively, at the level of their sequence, to kinases Cdk7 and Crk1, and to cyclin H and Mcs2 protein regulators had also been identified. The Kin28-Ccl1 complex was part of the TFIIH complex and has a CTD-kinase activity, but is not involved in the CAK activity.

Recently, the inventors have identified a kinase responsible for the CAK activity in *Saccharomyces cerevisiae*. This kinase has been designated CIV1 (CAK *in vivo*), and the corresponding gene has been designated *CIV1* (THURET et al., Cell, 86 (4), 1996). These results have been confirmed by other teams [KALDIS et al., Cell, 86 (4), 553-564 (1996); ESPINOZA et al., Science, 273 (5282), 1714-1717 (1996)]. The CAK of *Saccharomyces cerevisiae* is as a whole related to the serine-threonine-kinase family, and in particular to protein kinases CDC2 and CDC28, and is differentiated from the CAKs previously identified in other organisms by the absence of the glycine-rich conserved motif GxGx(Y/F)GxY, which is present in the majority of protein kinases, the presence of insertions of 5 to 29 amino acids, situated between elements of secondary structure conserved in the Cdk family, and by the fact that its CAK activity does not require its incorporation in an enzyme complex.

Since the CAK activity of CIV1 is ss ntial for survival and cell division, the inventors have und rtaken researching whether g n s homologous to *CIV1* exist in pathogenic yeasts, coding for protein kinases having a CAK activity. In fact, in this case, obtaining means for regulating this activity, and in particular, [finding] inhibitors, would attract great interest on the industrial or therapeutic plane, principally for obtaining fungicides.

With this objective, the inventors have first of all undertaken the screening of DNA databases for the pathogenic yeast *Candida albicans*, by using probes derived from different regions of the *CIV1* gene of *Saccharomyces cerevisiae*. However, none of the probes used permitted detecting the presence of homologous sequences in the *Candida albicans* genome.

The inventors have, however, researched also whether *Candida albicans* might have a functional analog of the CAK of *Saccharomyces cerevisiae*, by researching whether there is in *Candida albicans* a gene or genes capable of restoring the CAK function in *Saccharomyces cerevisiae* in a heat-sensitive mutant of the *CIV1* gene. They were thus able to identify a gene of *Candida albicans* capable of complementing by itself the deficient CAK function of the mutant.

The sequence of this gene, designated *CaCIV1* has been determined; it is shown in the list of appended sequences under number SEQ ID NO: 1; the sequence of its translation product, designated CaCIV1, is shown under the number SEQ ID NO: 2.

Figure 1 shows the comparison of the amino acid sequence (1-letter code) of CaCIV1 with that of the CAK of *Saccharomyces cerevisiae* (designated ScCIV1), and with that of the CDC28 kinase of *Saccharomyces cerevisiae* (designated ScCDC28). The residues conserved in ScCIV1 and CaCIV1 are indicated by bold letters.

Legend for the annotations of Figure 1:

k = residue conserved in the majority of protein kinases;

• = residue often present in the Cdk family;

o = residue always present in the Cdk family;

+ = residue present in the Cdk family and in ScCIV1;

Secondary structures: a = α helix; b = β sheet.

CaCIV1 only has an identity of 28% with the CAK of *Saccharomyces cerevisiae,* ScCIV1, at the level of the overall amino acid sequence.

Nevertheless, the similarities observed between ScCIV1 and CaCIV1 permit defining a family of kinases, hereinafter designated CIV1, regrouping proteins having the following characteristics:

- they lack the motif GxGx(Y/F)GxV, in which G indicates glycine, x represents any amino acid whatever, Y/F represents either tyrosine or phenylalanine, V represents valine;

- they have a non-cyclin dependent CAK activity.

The present invention includes the protein kinases belonging to the CIV1 family such as defined above, with the exception of CAK ScCIV1 of *Saccharomyces cerevisiae*.

According to a preferred mode of embodiment of the present invention, said protein kinase can be obtained from an Ascomycetes, advantageously a Hemiascomycetes, and preferably *Candida albicans*.

A protein kinase conforming to the invention is, for example, shown in the list of appended sequences under the number SEQ ID NO: 2.

The subject of the present invention is also a nucleic acid sequence coding for a protein kinase according to the invention.

A nucleic acid sequence conforming to the invention, for example, is constituted by the sequence SEQ ID NO: 1 of the list of appended sequences.

The subject of the present invention is also nucleic acid fragments of at least 18 bp, homologous or complementary to a nucleic acid sequence coding for a specific peptide sequence of the CAK conforming to the invention.

These fragments can be used, in particular, as hybridization probes, and/or amplification primers, to isolate and/or clone from *Candida albicans*, a nucleic acid sequence coding for a CAK conforming to the invention.

The present invention also includes nucleic acid fragments of at least 15 bp, preferably at least 18 bp, homologous or complementary to a nucleic acid sequence coding for a conserved peptide sequence in the protein kinase family defined by the CAK CaCIV1 conforming to the invention, and the CAK ScCIV1 of *Saccharomyces cerevisiae*.

These fragments can be used, in particular, as hybridization probes and/or amplification primers, to detect the existence, in organisms other than *Saccharomyces cerevisiae* and *Candida albicans*, of sequences coding for

kinases related to CAK CaCIV1 and ScCIV1 and to isolate and/or clone the genes thus identified. The invention also includes nucleic acid sequences obtained of this kind, and protein kinases of the CAK CaCIV1 and ScCIV1 family coded for by these sequences.

The subject of the present invention is also any recombinant vector, and in particular any expression vector, resulting from the insertion of at least one nucleic acid sequence conforming to the invention into an appropriate vector. An appropriate vector can be chosen easily by the person of average skill in the art, from among the numerous vectors available, depending on the host cell chosen to multiply and/or express a nucleic acid according to the invention.

The invention also includes prokaryotic or eukaryotic cells transformed by a nucleic acid sequence according to the invention. These transformed cells can be used in particular to express a kinase according to the invention, for example, in order to purify it from cell cultures, for example, by using techniques similar to those previously described by the inventors for CIV1 of *Saccharomyces cerevisiae*, (THURET et al., 1996, publication cited above), or also in order to detect its activity by an appropriate cell viability test.

The demonstration by the inventors of the functional homology of ScCIV1 and CaCIV1 kinases permits envisioning numerous applications for this family of kinases.

In particular, due to the fact that it appears that the non-cyclin dependent CAK activity of this family of kinases is essential for survival and cell division,

substances inhibiting this activity can be used as fungicid s, ith r as medications, or on the industrial plane.

For example, in order to screen fungicidal substances, such as substances active on *Candida albicans*, the kinase activity of CaCIV1, or one of its functional homologs comprised of a non-cyclin dependent CAK of the CIV1 family, is measured in the presence of each of the products for which one wishes to determine its fungicidal properties, and one selects the products having an inhibitor effect on this activity.

Such screening can be done by measuring the kinase activity of a CAK of the CIV1 family, in the presence of potential activators or inhibitors to be tested. The kinase activity, for example, can be measured *in vitro*, either directly by detecting the phosphorylation of a peptide or a protein substrate, for example, CDC28 or Cdk2, or the MBP protein (myelin basic protein), in an appropriate reaction mixture, or indirectly, by detecting and/or measuring the activity of the substrate protein when the latter depends on phosphorylation.

The kinase activity can also be measured *in vivo*, by a cell viability test; for example, the kinase activity of CaCIV1 can advantageously be measured in cells of a mutant of *Saccharomyces cerevisiae* not expressing the CAK ScCIV1, transformed by the *CaCIV1* gene.

The invention also includes the use of a product selected as indicated above for its inhibiting properties on a non-cyclin dependent CAK of the CIV1 family to obtain a fungicide.

The present invention will be better understood by means of the complementary description which will follow, which refers to an example illustrating the demonstration of the CAK activity of CaCIV1, and the cloning of the corresponding gene.

Example:

The strain of *Saccharomyces cerevisiae*, CMY975 (genotype *CIV1-2 ura3 leu2 trp1 lys2 ade2 ade3*) bears a heat-sensitive mutation of the *CIV1* gene and, as a result, grows at 24°C, but not at 37°C.

A culture of this strain was transformed by the lithium acetate method [SCHIESTL and GIETZ, Current Genetics, *16*, 339-346, (1989)] with a bank of Sau3A genomic fragments of *Candida albicans* cloned in the BamHI site of the YEp24 vector (multicopy-*URA3*) [Botstein et al., Gene *8*, (1979)].

The CMY975 cells are spread on boxes containing a synthetic medium devoid of uracil, and cultured at 37°C.

Ten colonies of CMY975 growing under these conditions were obtained. YEp24 plasmids containing *Candida albicans* inserts were recovered from each of these colonies and amplified in *Escherichia coli*.

The restriction map of each of these plasmids was established, and permitted observing that all the inserts originated from the same region of the genome of *Candida albicans*. The sequencing of this region permitted demonstrating the same open reading frame coding for a protein kinase of 339 amino acids, which shows that the 10 inserts obtained separately correspond to

on and the same gen of *Candida albicans*. This g ne was designated *CaCIV1*.

The *CaCIV1* sequence is shown in the list of appended sequences under the number SEQ ID NO: 1, and that of the CaCIV1 protein is shown under the number SEQ ID NO: 2. The comparison of the CaCIV1 protein sequence and the sequences available in the databases shows that this protein has only 28% amino acids identical with the CAK of *Saccharomyces cerevisiae* ScCIV1, and 24% amino acids identical with the Cdks ScCDC28 of *Saccharomyces cerevisiae* and CaCDC28 of *Candida albicans*.

A genomic BamHI-ClaI fragment of 3 kb containing the *CaCIV1* gene was subcloned in the centromeric plasmid TRP1 pRS414 [SIKORSKI and HIETER, Genetics, 122, 19-27 (1989)]. This plasmid was used for transforming a mutant of *Saccharomyces cerevisiae* in which the *ScCIV1* sequence is deleted from the genome and containing the *ScCIV1* gene on a replicative plasmid also bearing the selection gene *URA3* (strain CMY116 of the genome *ura3 leu2 trp1 lys2 civ1 LEU2/pJG43 (URA3-ScCIV1)*. The cells transformed with the plasmid pRS414-*CaCIVI* are viable after the counter-selection and the loss of the plasmid pJG43 (*URA-ScCIV1*), which confirms that the *CaCIV1* gene can function in the place of the essential *ScCIV1* gene. Therefore, *CaCIV1* codes for a functional homolog of *ScCIV1*, and suffices to restore the CAK activity.

CLAIMS

1. A protein kinase, belonging to the family designated CIV1 defined by the following characteristics:

- it lacks the motif GxGx(Y/F)GxV, in which G represents glycine, x represents any amino acid whatever, Y/F represents either tyrosine or phenylalanine, V represents valine;

- it has a non-cyclin dependent CAK activity;

with the exception of the CAK ScCIV1 of *Saccharomyces cerevisiae*.

2. The protein kinase according to claim 1, further characterized in that it can be obtained from *Candida albicans*.

3. The protein kinase according to either one of claims 1 or 2, further characterized in that it conforms to the sequence represented in the list of appended sequences under the number SEQ ID NO: 2.

4. A nucleic acid coding for a protein kinase according to any one of claims 1 to 3.

5. A recombinant vector, resulting from the insertion of a nucleic acid according to claim 4 into an appropriate vector.

6. A method for screening fungicidal products, characterized in that it comprises a step where the kinase activity of a non-cyclin dependent CAK of the CIV1 family defined in claim 1 is measured in the presence of each

of the products for which one wishes to determine the fungicidal properties, and the products having an inhibitor effect on this activity are selected.

7. Use of a product selected by the method according to claim 6 for obtaining a fungicide.